

# A Petri net approach to the study of persistence in chemical reaction networks

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## Abstract

Persistency is the property, for differential equations in  $\mathbb{R}^n$ , that solutions starting in the positive orthant do not approach the boundary. For chemical reactions and population models, this translates into the non-extinction property: provided that every species is present at the start of the reaction, no species will tend to be eliminated in the course of the reaction. This paper provides checkable conditions for persistence of chemical species in reaction networks, using concepts and tools from Petri net theory, and verifies these conditions on various systems which arise in the modeling of cell signaling pathways.

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# 1 Introduction

One of the main goals of molecular systems biology is the understanding of cell behavior and function at the level of chemical interactions, and, in particular, the characterization of qualitative features of dynamical behavior (convergence to steady states, periodic orbits, chaos, etc). A central question, thus, is that of understanding the long-time behavior of solutions. In mathematical terms, and using standard chemical kinetics modeling, this problem may be translated into the study of the set of possible limit points (the  $\omega$ -limit set) of the solutions of a system of ordinary differential equations.

## Robustness

A distinguishing feature of this study in the context of cell biology, in contrast to more established areas of applied mathematics and engineering, is the very large degree of uncertainty inherent in models of cellular biochemical networks. This uncertainty is due to environmental fluctuations, and variability among different cells of the same type, as well as, from a mathematical analysis perspective, the difficulty of measuring the relevant model parameters (kinetic constants, cooperativity indices, and many others) and thus the impossibility of obtaining a precise model. Thus, it is imperative to develop tools that are “robust” in the sense of being able to provide useful conclusions based only upon information regarding the *qualitative* features of the network, and not the precise values of parameters or even the forms of reactions. Of course, this goal is often not unachievable, since dynamical behavior may be subject to phase transitions (bifurcation phenomena) which are critically dependent on parameter values.

Nevertheless, and surprisingly, research by many, notably by Clarke [10], Horn and Jackson [22, 23], Feinberg [13, 14, 15], and many others in the context of complex balancing and deficiency theory, and by Hirsch and Smith [33, 19] and many others including the present authors [2, 12, 3, 9] in the context of monotone systems, has resulted in the identification of rich classes of chemical network structures for which such robust analysis is indeed possible. In this paper, we present yet another set of tools for the robust analysis of dynamical properties of biochemical networks, and apply our approach in particular to the analysis of persistence in chemical networks modeled by ordinary differential equations. Our approach to studying persistence is based on the formalism and basic concepts of the theory of Petri nets. Using these techniques, our main results provide conditions (some necessary, and some sufficient) to test persistence. We then apply these conditions to obtain fairly tight characterizations in non-trivial examples arising from the current molecular biology literature.

## Persistence

*Persistence* is the property that, *if every species is present at the start of the reaction, no species will tend to be eliminated in the course of the reaction*. Mathematically, this property can be equivalently expressed as the requirement that the  $\omega$ -limit set of any

trajectory which starts in the interior of the positive orthant (all concentrations positive) does not intersect the boundary of the positive orthant (more precise definitions are given below). Persistency can be interpreted as non-extinction: if the concentration of a species would approach zero in the continuous differential equation model, this means, in practical terms, that it would completely disappear in finite time, since the true system is discrete and stochastic. Thus, one of the most basic questions that one may ask about a chemical reaction is if persistency holds for that network. Also from a purely mathematical perspective persistency is very important, because it may be used in conjunction with other tools in order to guarantee convergence of solutions to equilibria. For example, if a strictly decreasing Lyapunov function exists on the interior of the positive orthant (see e.g. [22, 23, 13, 14, 15, 34] for classes of networks where this can be guaranteed), persistency allows such a conclusion.

An obvious example of a non-persistent chemical reaction is a simple irreversible conversion  $A \rightarrow B$  of a species  $A$  into a species  $B$ ; in this example, the chemical  $A$  empties out, that is, its time-dependent concentration approaches zero as  $t \rightarrow \infty$ . This is obvious, but for complex networks determining persistency, or lack thereof, is, in general, an extremely difficult mathematical problem. In fact, the study of persistence is a classical one in the (mathematically) related field of population biology, where species correspond to individuals of different types instead of chemical units; see for example [17, 7] and much other foundational work by Waltman. (To be precise, what we call “persistence” coincides with the usage in the above references, and is also sometimes called “strong persistence,” at least when all solutions are bounded, a condition that we will assume in most of our main results, and which is automatically satisfied in most examples. Also, we note that a stronger notion, “uniform” persistence, is used to describe the situation where all solutions are eventually bounded away from the boundary, uniformly on initial conditions, see [8, 36].) Most dynamical systems work on persistence imposes conditions ruling out phenomena such as heteroclinic cycles on the boundary of the positive orthant, and requiring that the unstable manifolds of boundary equilibria should intersect the interior, and more generally studying the chain-recurrence structure of attractors, see e.g. [20].

## Petri nets

*Petri nets*, also called place/transition nets, were introduced by Carl Adam Petri in 1962 [31], and they constitute a popular mathematical and graphical modeling tool used for concurrent systems modeling [30, 39]. Our modeling of chemical reaction networks using Petri net formalism is not in itself a new idea: there have been many works, at least since [32], which have dealt with biochemical applications of Petri nets, in particular in the context of metabolic pathways, see e.g. [18, 21, 25, 28, 29], and especially the excellent exposition [38]. However, there does not appear to have been any previous work using Petri nets for a nontrivial study of dynamics. In this paper, although we do not use any results from Petri net theory, we employ several concepts (siphons, locking sets, etc.), borrowed from that formalism and introduced as needed, in order to formulate

new, powerful, and verifiable conditions for persistence and related dynamical properties.

### Application to a common motif in systems biology

In molecular systems biology research, certain “motifs” or subsystems appear repeatedly, and have been the subject of much recent research. One of the most common ones is that in which a substrate  $S_0$  is ultimately converted into a product  $P$ , in an “activation” reaction triggered or facilitated by an enzyme  $E$ , and, conversely,  $P$  is transformed back (or “deactivated”) into the original  $S_0$ , helped on by the action of a second enzyme  $F$ . This type of reaction is sometimes called a “futile cycle” and it takes place in signaling transduction cascades, bacterial two-component systems, and a plethora of other processes. The transformations of  $S_0$  into  $P$  and vice versa can take many forms, depending on how many elementary steps (typically phosphorylations, methylations, or additions of other elementary chemical groups) are involved, and in what order they take place. Figure 1 shows two examples, (a) one in which a single step takes place changing  $S_0$  into  $P = S_1$ , and (b) one in which two sequential steps are needed to transform  $S_0$  into  $P = S_2$ , with an intermediate transformation into a substance  $S_1$ . A chemical reaction model for such

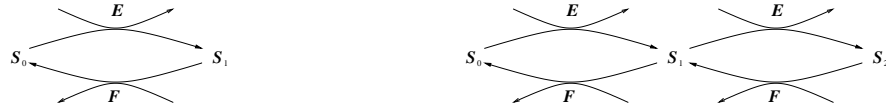
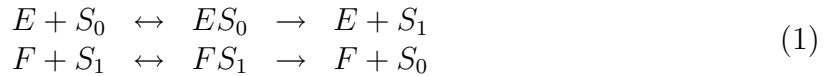


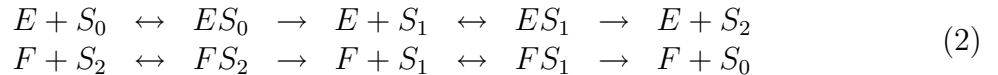
Figure 1: (a) *One-step*

and (b) *two-step transformations*

a set of transformations incorporates intermediate species, compounds corresponding to the binding of the enzyme and substrate. (In “quasi-steady state” approximations, a singular perturbation approach is used in order to eliminate the intermediates. These approximations are much easier to study, see e.g. [2].) Thus, one model for (a) would be through the following reaction network:



(double arrows indicate reversible reactions) and a model for (b) would be:



where “ $ES_0$ ” represents the complex consisting of  $E$  bound to  $S_0$  and so forth.

As a concrete example, case (b) may represent a reaction in which the enzyme  $E$  reversibly adds a phosphate group to a certain specific amino acid in the protein  $S_0$ , resulting in a single-phosphorylated form  $S_1$ ; in turn,  $E$  can then bind to  $S_1$  so as to produce a double-phosphorylated form  $S_2$ , when a second amino acid site is phosphorylated. A different enzyme reverses the process. (Variants in which the individual phosphorylations can occur in different orders are also possible; we discuss several models below.)

This is, in fact, one of the mechanisms believed to underlie signaling by MAPK cascades. *Mitogen-activated protein kinase (MAPK) cascades* constitute a motif that is ubiquitous in signal transduction processes [24, 26, 37] in eukaryotes from yeast to humans, and represents a critical component of pathways involved in cell apoptosis, differentiation, proliferation, and other processes. These pathways involve chains of reactions, activated by extracellular stimuli such as growth factors or hormones, and resulting in gene expression or other cellular responses. In MAPK cascades, several steps as in (b) are arranged in a cascade, with the “active” form  $S_2$  serving as an enzyme for the next stage.

Single-step reactions as in (a) can be shown to have the property that all solutions starting in the interior of the positive orthant globally converge to a unique (subject to stoichiometry constraints) steady state, see [4], and, in fact, can be modeled by monotone systems after elimination of the variables  $E$  and  $F$ , cf. [1]. The study of (b) is much harder, as multiple equilibria can appear, see e.g. [27, 11]. We will show how our results can be applied to test consistency of this model, as well as several variants.

## Organization of paper

The remainder of paper is organized as follows. Section 2 sets up the basic terminology and definitions regarding chemical networks, as well as the notion of persistence, Section 3 shows how to associate a Petri net to a chemical network, Sections 4 and 5 provide, respectively, necessary and sufficient conditions for general chemical networks, In Section 6, we show how our results apply to the enzymatic mechanisms described above.

## 2 Chemical Networks

A *chemical reaction network* (“CRN”, for short) is a set of chemical reactions  $\mathcal{R}_i$ , where the index  $i$  takes values in  $\mathcal{R} := \{1, 2, \dots, n_r\}$ . We next define precisely what one means by reactions, and the differential equation associated to a CRN, using the formalism from chemical networks theory.

Let us consider a set of chemical species  $S_j$ ,  $j \in \{1, 2, \dots, n_s\} := \mathcal{S}$  which are the compounds taking part in the reactions. Chemical reactions are denoted as follows:

$$\mathcal{R}_i : \quad \sum_{j \in \mathcal{S}} \alpha_{ij} S_j \rightarrow \sum_{j \in \mathcal{S}} \beta_{ij} S_j \quad (3)$$

where the  $\alpha_{ij}$  and  $\beta_{ij}$  are nonnegative integers called the *stoichiometry coefficients*. The compounds on the left-hand side are usually referred to as the *reactants*, and the ones on the right-hand side are called the *products*, of the reaction. Informally speaking, the forward arrow means that the transformation of reactants into products only happens in the direction of the arrow. If also the converse transformation occurs, then, the reaction is reversible and we need to also list its inverse in the chemical reaction network as a separate reaction.

It is convenient to arrange the stoichiometry coefficients into an  $n_s \times n_r$  matrix, called the *stoichiometry matrix*  $\Gamma$ , defined as follows:

$$[\Gamma]_{ji} = \beta_{ij} - \alpha_{ij}, \quad (4)$$

for all  $i \in \mathcal{R}$  and all  $j \in \mathcal{S}$  (notice the reversal of indices). This will be later used in order to write down the differential equation associated to the chemical reaction network. Notice that we allow  $\Gamma$  to have columns which differ only by their sign; this happens when there are reversible reactions in the network.

We discuss now how the speed of reactions is affected by the concentrations of the different species. Each chemical reaction takes place continuously in time with its own rate which is assumed to be only a function of the concentration of the species taking part in it. In order to make this more precise, we define the vector  $S = [S_1, S_2, \dots, S_{n_s}]'$  of species concentrations and, as a function of it, the vector of reaction rates

$$R(S) := [R_1(S), R_2(S), \dots, R_{n_r}(S)]'.$$

Each reaction rate  $R_i$  is a real-analytic function defined on an open set which contains the non-negative orthant  $\mathcal{O}_+ = \mathbb{R}_{\geq 0}^{n_s}$  of  $\mathbb{R}^{n_s}$ , and we assume that each  $R_i$  depends only on its respective reactants. (Imposing real-analyticity, that is to say, that the function  $R_i$  can be locally expanded into a convergent power series around each point in its domain, is a very mild assumption, verified in basically all applications in chemistry, and it allows stronger statements to be made.) Furthermore, we assume that each  $R_i$  satisfies the following monotonicity conditions:

$$\frac{\partial R_i(S)}{\partial S_j} = \begin{cases} \geq 0 & \text{if } \alpha_{ij} > 0 \\ = 0 & \text{if } \alpha_{ij} = 0. \end{cases} \quad (5)$$

We also assume that, whenever the concentration of any of the reactants of a given reaction is 0, then, the corresponding reaction does not take place, meaning that the reaction rate is 0. In other words, if  $S_{i_1}, \dots, S_{i_N}$  are the reactants of reaction  $j$ , then we ask that

$$R_j(S) = 0 \text{ for all } S \text{ such that } [S_{i_1}, \dots, S_{i_N}] \in \partial\mathcal{O}_+,$$

where  $\partial\mathcal{O}_+ = \partial\mathbb{R}_{\geq 0}^N$  is the boundary of  $\mathcal{O}_+$  in  $\mathbb{R}^N$ . Conversely, we assume that reactions take place if reactants are available, that is:

$$R_j(S) > 0 \text{ whenever } S \text{ is such that } [S_{i_1}, \dots, S_{i_N}] \in \text{int}[\mathbb{R}_{\geq 0}^N].$$

A special case of reactions is as follows. One says that a chemical reaction network is equipped with *mass-action kinetics* if

$$R_i(S) = k_i \prod_{j=1}^{n_s} S_j^{\alpha_{ij}} \text{ for all } i = 1, \dots, n_r.$$

This is a commonly used form for the functions  $R_i(s)$  and amounts to asking that the reaction rate of each reaction is proportional to the concentration of each of its participating reactants. The results in this paper do not require this assumption; in a paper in preparation we will specialize and tighten our results when applied to systems with mass-action kinetics.

With the above notations, the chemical reaction network is described by the following system of differential equations:

$$\dot{S} = \Gamma R(S). \quad (6)$$

with  $S$  evolving in  $\mathcal{O}_+$  and where  $\Gamma$  is the stoichiometry matrix.

There are several additional notions useful when analyzing CRN's. One of them is the notion of a *complex*. We associate to the network (3) a set of complexes,  $C_i$ 's, with  $i \in \{1, 2, \dots, n_c\}$ . Each complex is an integer combination of species, specifically of the species appearing either as products or reactants of the reactions in (3). We introduce the following matrix  $\tilde{\Gamma}$  as follows:

$$\tilde{\Gamma} = \begin{bmatrix} \alpha_{11} & \alpha_{21} & \dots & \alpha_{n_r 1} & \beta_{11} & \beta_{21} & \dots & \beta_{n_r 1} \\ \alpha_{12} & \alpha_{22} & \dots & \alpha_{n_r 2} & \beta_{12} & \beta_{22} & \dots & \beta_{n_r 2} \\ \vdots & \vdots & & \vdots & \vdots & \vdots & & \vdots \\ \alpha_{1n_s} & \alpha_{2n_s} & \dots & \alpha_{n_r n_s} & \beta_{1n_s} & \beta_{2n_s} & \dots & \beta_{n_r n_s} \end{bmatrix}$$

Then, a matrix representing the complexes as columns can be obtained by deleting from  $\tilde{\Gamma}$  repeated columns, leaving just one instance of each; we denote by  $\Gamma_c \in \mathbb{R}^{n_s \times n_c}$  the matrix which is thus constructed. Each of the columns of  $\Gamma_c$  is then associated with a complex of the network. We may now associate to each chemical reaction network, a directed graph (which we call the *C-graph*), whose nodes are the complexes and whose edges are associated to the reactions (3). An edge  $(C_i, C_j)$  is in the C-graph if and only if  $C_i \rightarrow C_j$  is a reaction of the network. Note that the C-graph need not be connected (the C-graph is connected if for any pair of distinct nodes in the graph there is an undirected path linking the nodes), and lack of connectivity cannot be avoided in the analysis. (This is in contrast with many other graphs in chemical reaction theory, which can be assumed to be connected without loss of generality.) In general, the C-graph will have several connected components (equivalence classes under the equivalence relation “is linked by an undirected path to”, defined on the set of nodes of the graph).

Let  $\mathcal{I}$  be the incidence matrix of the C-graph, namely the matrix whose columns are in one-to-one correspondence with the edges (reactions) of the graph and whose rows are in one-to-one correspondence with the nodes (complexes). Each column contains a  $-1$  in the  $i$ -th entry and a  $+1$  in the  $j$ -th entry (and zeroes in all remaining entries) whenever  $(C_i, C_j)$  is an edge of the C-graph (equivalently, when  $C_i \rightarrow C_j$  is a reaction of the network). With this notations, we have the following formula, to be used later:

$$\Gamma = \Gamma_c \mathcal{I}. \quad (7)$$

We denote solutions of (6) as follows:  $S(t) = \varphi(t, S_0)$ , where  $S_0 \in \mathcal{O}_+$  is the initial concentration of chemical species. As usual in the study of the qualitative behavior

of dynamical systems, we will make use of  $\omega$ -limit sets, which capture the long-term behavior of a system and are defined as follows:

$$\omega(S_0) := \{S \in \mathcal{O}_+ : \varphi(t_n, S_0) \rightarrow S \text{ for some } t_n \rightarrow +\infty\} \quad (8)$$

(implicitly, when talking about  $\omega(S_0)$ , we assume that  $\varphi(t, S_0)$  is defined for all  $t \geq 0$  for the initial condition  $S_0$ ). We will be interested in asking whether or not a chemical reaction network admits solutions in which one or more of the chemical compounds become arbitrarily small. The following definition, borrowed from the ecology literature, captures this intuitive idea.

**Definition 2.1** A chemical reaction network (6) is *persistent* if  $\omega(S_0) \cap \partial\mathcal{O}_+ = \emptyset$  for each  $S_0 \in \text{int}(\mathcal{O}_+)$ .  $\square$

We will derive conditions for persistence of general chemical reaction networks. Our conditions will be formulated in the language of Petri nets; these are discrete-event systems equipped with an algebraic structure that reflects the list of chemical reactions present in the network being studied, and are defined as follows.

### 3 Petri Nets

We associate to a CRN a bipartite directed graph (i.e., a directed graph with two types of nodes) with weighted edges, called the *species-reaction Petri net*, or SR-net for short. Mathematically, this is a quadruple

$$(V_S, V_R, E, W),$$

where  $V_S$  is a finite set of nodes each one associated to a species,  $V_R$  is a finite set of nodes (disjoint from  $V_S$ ), each one corresponding to a reaction, and  $E$  is a set of edges as described below. (We often write  $S$  or  $V_S$  interchangeably, or  $R$  instead of  $V_R$ , by identifying species or reactions with their respective indices; the context should make the meaning clear.) The set of all nodes is also denoted by  $V \doteq V_R \cup V_S$ .

The edge set  $E \subset V \times V$  is defined as follows. Whenever a certain reaction  $R_i$  belongs to the CRN:

$$\sum_{j \in \mathcal{S}} \alpha_{ij} S_j \rightarrow \sum_{j \in \mathcal{S}} \beta_{ij} S_j, \quad (9)$$

we draw an edge from  $S_j \in V_S$  to  $R_i \in V_R$  for all  $S_j$ 's such that  $\alpha_{ij} > 0$ . That is,  $(S_j, R_i) \in E$  iff  $\alpha_{ij} > 0$ , and we say in this case that  $R_i$  is an *output reaction* for  $S_j$ . Similarly, we draw an edge from  $R_i \in V_R$  to every  $S_j \in V_S$  such that  $\beta_{ij} > 0$ . That is,  $(R_i, S_j) \in E$  whenever  $\beta_{ij} > 0$ , and we say in this case that  $R_i$  is an *input reaction* for  $S_j$ .

Notice that edges only connect species to reactions and vice versa, but never connect two species or two reactions.



The last element to fully define the Petri net is the function  $W : E \rightarrow \mathbb{N}$ , which associates to each edge a positive integer according to the rule:

$$W(S_j, R_i) = \alpha_{ij} \quad \text{and} \quad W(R_i, S_j) = \beta_{ij}.$$

Several other definitions which are commonly used in the Petri net literature will be of interest in the following. We say that a row or column vector  $v$  is non-negative, and we denote it by  $v \succeq 0$  if it is so entry-wise. We write  $v \succ 0$  if  $v \succeq 0$  and  $v \neq 0$ . A stronger notion is instead  $v \gg 0$ , which indicates  $v_i > 0$  for all  $i$ .

**Definition 3.1** A *P-semiflow* is any row vector  $c \succ 0$  such that  $c\Gamma = 0$ . Its *support* is the set of indices  $\{i \in V_S : c_i > 0\}$ . A Petri net is said to be *conservative* if there exists a P-semiflow  $c \gg 0$ .  $\square$

Notice that P-semiflows for the system (6) correspond to non-negative linear first integrals, that is, linear functions  $S \mapsto cS$  such that  $(d/dt)cS(t) \equiv 0$  along all solutions of (6) (assuming that the span of the image of  $R(S)$  is  $\mathbb{R}^{n_r}$ ). In particular, a Petri net is conservative if and only if there is a positive linear conserved quantity for the system. (Petri net theory views Petri nets as “token-passing” systems, and, in that context, P-semiflows, also called *place-invariants*, amount to conservation relations for the “place markings” of the network, that show how many tokens there are in each “place,” the nodes associated to species in SR-nets. We do not make use of this interpretation in this paper.)

**Definition 3.2** A *T-semiflow* is any column vector  $v \succ 0$  such that  $\Gamma v = 0$ . A Petri net is said to be *consistent* if there exists a T-semiflow  $v \gg 0$ .  $\square$

The notion of T-semiflow corresponds to the existence of a collection of positive reaction rates which do not produce any variation in the concentrations of the species. In other words,  $v$  can be viewed as a set of *fluxes* that is in equilibrium ([38]). (In Petri net theory, the terminology is “T-invariant,” and the fluxes are flows of tokens.)

A chemical reaction network is said to be *reversible* if each chemical reaction has an inverse reaction which is also part of the network. Biochemical models are most often non-reversible. For this reason, a far milder notion was introduced [22, 23, 13, 14, 15]: A chemical reaction network is said to be *weakly reversible* if each connected component of the C-graph is strongly connected (meaning that there is a directed path between any pair of nodes in each connected component). In algebraic terms, weak reversibility amounts to existence of  $v \gg 0$  such that  $\mathcal{I}v = 0$  (see Corollary 4.2 of [16]), so that in particular, using (7), also  $\Gamma v = \Gamma \mathcal{I}v = 0$ . Hence a chemical reaction network that is weakly reversible has a consistent associated Petri net.

A few more definitions are needed in order to state our main results.

**Definition 3.3** A nonempty set  $\Sigma \subset V_S$  is called a *siphon* if each input reaction associated to  $\Sigma$  is also an output reaction associated to  $\Sigma$ . A siphon is a *deadlock* if its set of output reactions is all of  $V_R$ . A deadlock is *minimal* if it does not contain (strictly) any other deadlocks. A pair of distinct deadlocks  $\Sigma_1$  and  $\Sigma_2$  is said to be *nested* if either  $\Sigma_1 \subset \Sigma_2$  or  $\Sigma_2 \subset \Sigma_1$ .  $\square$

For later use we associate a particular set to a siphon  $\Sigma$  as follows:

$$L_\Sigma = \{x \in \mathcal{O}_+ \mid x_i = 0 \iff i \in \Sigma\}.$$

It is also useful to introduce a binary relation “reacts to”, which we denote by  $\succrightarrow$ , and we define as follows:  $S_i \succrightarrow S_j$  whenever there exists a chemical reaction  $\mathcal{R}_k$ , so that

$$\sum_{l \in \mathcal{S}} \alpha_{kl} S_l \rightarrow \sum_{l \in \mathcal{S}} \beta_{kl} S_l$$

with  $\alpha_{ki} > 0$ ,  $\beta_{kj} > 0$ . If the reaction number is important, we also write

$$S_i \xrightarrow{k} S_j$$

(where  $k \in \mathcal{R}$ ). With this notation, the notion of siphon can be rephrased as follows:  $Z \subset \mathcal{S}$  is a siphon for a chemical reaction network if for every  $S \in Z$  and  $k \in \mathcal{R}$  such that  $\tilde{S}_k := \{T \in \mathcal{S} : T \xrightarrow{k} S\} \neq \emptyset$ , it holds  $\tilde{S}_k \cap Z \neq \emptyset$ .

## 4 Necessary conditions

Our first result will relate persistence of a chemical reaction network to consistency of the associated Petri net.

**Theorem 1** *Let (6) be the equation describing the time-evolution of a conservative and persistent chemical reaction network. Then, the associated Petri net is consistent.*

*Proof.* Let  $S_0 \in \text{int}(\mathcal{O}_+)$  be any initial condition. By conservativity, solutions satisfy  $cS(t) \equiv cS_0$ , and hence remain bounded, and therefore  $\omega(S_0)$  is a nonempty compact set. Moreover, by persistence,  $\omega(S_0) \cap \partial\mathcal{O}_+ = \emptyset$ , so that  $R(\tilde{S}_0) \gg 0$ , for all  $\tilde{S}_0 \in \omega(S_0)$ . In particular, by compactness of  $\omega(S_0)$  and continuity of  $R$ , there exists a positive vector  $v \gg 0$ , so that

$$R(\tilde{S}_0) \succeq v \text{ for all } \tilde{S}_0 \in \omega(S_0).$$

Take any  $\tilde{S}_0 \in \omega(S_0)$ . By invariance of  $\omega(S_0)$ , we have  $R(\varphi(t, \tilde{S}_0)) \succeq v$  for all  $t \in \mathbb{R}$ . Consequently, taking asymptotic time averages, we obtain:

$$0 = \lim_{T \rightarrow +\infty} \frac{\varphi(T, \tilde{S}_0) - \tilde{S}_0}{T} = \lim_{T \rightarrow +\infty} \frac{1}{T} \int_0^T \Gamma R(\varphi(t, \tilde{S}_0)) dt \quad (10)$$

(the left-hand limit is zero because  $\varphi(T, \tilde{S}_0)$  is bounded). However,

$$\frac{1}{T} \int_0^T R(\varphi(t, \tilde{S}_0)) dt \succeq v$$

for all  $T > 0$ . Therefore, taking any subsequence  $T_n \rightarrow +\infty$  so that there is a finite limit:

$$\lim_{n \rightarrow +\infty} \frac{1}{T_n} \int_0^{T_n} R(\varphi(t, \tilde{S}_0)) dt = \bar{v} \succeq v.$$

We obtain, by virtue of (10), that  $\Gamma \bar{v} = 0$ . This completes the proof of consistency, since  $\bar{v} \gg 0$ . ■

## 5 Sufficient Conditions

In this present Section, we derive sufficient conditions for insuring persistence of a chemical reaction network on the basis of Petri net properties.

**Theorem 2** *Consider a chemical reaction network satisfying the following assumptions:*

1. *its associated Petri net is conservative;*
2. *each siphon contains the support of a P-semiflow.*

*Then, the network is persistent.*

We first prove a number of technical results. The following general fact about differential equations will be useful.

For each real number  $p$ , let  $\text{sign } p := 1, 0, -1$  if  $p > 0$ ,  $p = 0$ , or  $p < 0$  respectively, and for each vector  $x = (x_1, \dots, x_n)$ , let  $\text{sign } x := (\text{sign } x_1, \dots, \text{sign } x_n)'$ . When  $x$  belongs to the closed positive orthant  $\mathbb{R}_+^n$ ,  $\text{sign } x \in \{0, 1\}^n$ .

**Lemma 5.1** Let  $f$  be a real-analytic vector field defined on some open neighborhood of  $\mathbb{R}_+^n$ , and suppose that  $\mathbb{R}_+^n$  is forward invariant for the flow of  $f$ . Consider any solution  $\bar{x}(t)$  of  $\dot{x} = f(x)$ , evolving in  $\mathbb{R}_+^n$  and defined on some open interval  $J$ . Then,  $\text{sign } \bar{x}(t)$  is constant on  $J$ .

*Proof.* Pick such a solution, and define

$$Z := \{i \mid \bar{x}_i(t) = 0 \text{ for all } t \in J\}.$$

Relabeling variables if necessary, we assume without loss of generality that  $Z = \{r + 1, \dots, n\}$ , with  $0 \leq r \leq n$ , and we write equations in the following block form:

$$\begin{aligned}\dot{y} &= g(y, z) \\ \dot{z} &= h(y, z)\end{aligned}$$

where  $x' = (y', z')'$  and  $y(t) \in \mathbb{R}^r$ ,  $z(t) \in \mathbb{R}^{n-r}$ . (The extreme cases  $r = 0$  and  $r = n$  correspond to  $x = z$  and  $x = y$  respectively.) In particular, we write  $\bar{x}' = (\bar{y}', \bar{z}')'$  for the trajectory of interest. By construction,  $\bar{z} \equiv 0$ , and the sets

$$B_i := \{t \mid \bar{y}_i(t) = 0\}$$

are proper subsets of  $J$ , for each  $i \in \{1, \dots, r\}$ . Since the vector field is real-analytic, each coordinate function  $\bar{y}_i$  is real-analytic (see e.g. [35], Proposition C.3.12), so, by the principle of analytic continuation, each  $B_i$  is a discrete set. It follows that

$$G := J \setminus \bigcup_{i=1}^r B_i$$

is an (open) dense set, and for each  $t \in G$ ,  $\bar{y}(t) \in \text{inter } \mathbb{R}_+^r$ , the interior of the positive orthant.

We now consider the following system on  $\mathbb{R}^r$ :

$$\dot{y} = g(y, 0).$$

This is again a real-analytic system, and  $\mathbb{R}_+^r$  is forward invariant. To prove this last assertion, note that forward invariance of the closed positive orthant is equivalent to the following property:

for any  $y \in \mathbb{R}_+^r$  and any  $i \in \{1, \dots, r\}$  such that  $y_i = 0$ ,  $g_i(y, 0) \geq 0$ .

Since  $\mathbb{R}_+^n$  is forward invariant for the original system, we know, by the same property applied to that system, that for any  $(y, z) \in \mathbb{R}_+^n$  and any  $i \in \{1, \dots, r\}$  such that  $y_i = 0$ ,  $g_i(y, z) \geq 0$ . Thus, the required property holds (case  $z = 0$ ). In particular,  $\text{inter } \mathbb{R}_+^r$  is also forward invariant (see e.g. [2], Lemma III.6). By construction,  $\bar{y}$  is a solution of  $\dot{y} = g(y, 0)$ ,  $\bar{y}(t) \in \text{inter } \mathbb{R}_+^r$  for each  $t \in G$ . Since  $G$  is dense and  $\text{inter } \mathbb{R}_+^r$  is forward invariant, it follows that  $\bar{y}(t) \in \text{inter } \mathbb{R}_+^r$  for all  $t \in J$ . Therefore,

$$\text{sign } \bar{x}(t) = (1_r, 0_{n-r})' \text{ for all } t \in J$$

where  $1_r$  is a vector of  $r$  1's and  $0_{n-r}$  is a vector of  $n - r$  0's. ■

We then have an immediate corollary:

**Lemma 5.2** Suppose that  $\Omega \subset \mathcal{O}_+$  is a closed set, invariant for (6). Suppose that  $\Omega \cap L_Z$  is non-empty, for some  $Z \subset \mathcal{S}$ . Then,  $\Omega \cap L_Z$  is also invariant with respect to (6).

*Proof.* Pick any  $S_0 \in \Omega \cap L_Z$ . By invariance of  $\Omega$ , the solution  $\varphi(t, S_0)$  belongs to  $\Omega$  for all  $t$  in its open domain of definition  $J$ , so, in particular (this is the key fact),  $\varphi(t, S_0) \in \mathcal{O}_+$  for all  $t$  (negative as well as positive). Therefore, it also belongs to  $L_Z$ , since its sign is constant by Lemma 5.1. ■

In what follows, we will make use of the Bouligand tangent cone  $TC_\xi(K)$  of a set  $K \subset \mathcal{O}_+$  at a point  $\xi \in \mathcal{O}_+$ , defined as follows:

$$TC_\xi(K) = \left\{ v \in \mathbb{R}^n : \exists k_n \in K, k_n \rightarrow \xi \text{ and } \lambda_n \searrow 0 : \frac{1}{\lambda_n}(k_n - \xi) \rightarrow v \right\}.$$

Bouligand cones provide a simple criterion to check forward invariance of closed sets (see e.g. [5]): a closed set  $K$  is forward invariant for (6) if and only if  $\Gamma R(\xi) \in TC_\xi(K)$  for all  $\xi \in K$ . However, below we consider a condition involving tangent cones to the sets  $L_Z$ , which are not closed. Note that, for all index sets  $Z$  and all points  $\xi$  in  $L_Z$ ,

$$TC_\xi(L_Z) = \{v \in \mathbb{R}^n : v_i = 0 \ \forall i \in Z\}.$$

**Lemma 5.3** Let  $Z \subset \mathcal{S}$  be non-empty and  $\xi \in L_Z$  be such that  $\Gamma R(\xi) \in TC_\xi(L_Z)$ . Then  $Z$  is a *siphon*.

*Proof.* By assumption  $\Gamma R(\xi) \in TC_\xi(L_Z)$  for some  $\xi \in L_Z$ . This implies that  $[\Gamma R(\xi)]_i = 0$  for all  $i \in Z$ . Since  $\xi_i = 0$  for all  $i \in Z$ , all reactions in which  $S_i$  is involved as a reactant are shut off at  $\xi$ ; hence, the only possibility for  $[\Gamma R(\xi)]_i = 0$  is that all reactions in which  $S_i$  is involved as a product are also shut-off. Hence, for all  $k \in \mathcal{R}$ , and all  $l \in \mathcal{S}$  so that  $S_l \xrightarrow{k} S_i$ , we necessarily have that  $R_k(\xi) = 0$ .

Hence, for all  $k \in \mathcal{R}$  so that  $\tilde{S}_k = \{l \in \mathcal{S} : S_l \xrightarrow{k} S_i\}$  is non-empty, there must exist an  $l \in \tilde{S}_k$  so that  $\xi_l = 0$ . But then necessarily,  $l \in Z$ , showing that  $Z$  is indeed a siphon. ■

The above Lemmas are instrumental to proving the following Proposition:

**Proposition 5.4** Let  $\xi \in \mathcal{O}^+$  be such that  $\omega(\xi) \cap L_Z \neq \emptyset$  for some  $Z \subset \mathcal{S}$ . Then  $Z$  is a siphon.

*Proof.* Let  $\Omega$  be the closed and invariant set  $\omega(\xi)$ . Thus, by Lemma 5.2, the non-empty set  $L_Z \cap \Omega$  is also invariant. Notice that

$$\text{cl}[L_Z] = \bigcup_{W \supseteq Z} L_W.$$

Moreover,  $L_W \cap \Omega$  is invariant for all  $W \subset \mathcal{S}$  such that  $L_W \cap \Omega$  is non-empty. Hence,

$$\text{cl}[L_Z] \cap \Omega = \bigcup_{W \supseteq Z} [L_W \cap \Omega]$$

is also invariant. By the characterization of invariance for closed sets in terms of Bouligand tangent cones, we know that, for any  $\eta \in \text{cl}[L_Z] \cap \Omega$  we have

$$\Gamma R(\eta) \in TC_\eta(\Omega \cap \text{cl}(L_Z)) \subset TC_\eta(\text{cl}(L_Z)).$$

In particular, for  $\eta \in L_Z \cap \Omega$  (which by assumption exists),  $\Gamma R(\eta) \in TC_\eta(L_Z)$  so that, by virtue of Lemma 5.3 we may conclude  $Z$  is a siphon.  $\blacksquare$

Although at this point Proposition 5.4 would be enough to prove Theorem 2, it is useful to clarify the meaning of the concept of a “siphon” here. It hints at the fact, made precise in the Proposition below, that removing all the species of a siphon from the network (or equivalently setting their initial concentrations equal to 0) will prevent those species from being present at all future times. Hence, those species literally “lock” a part of the network and shut off all the reactions that are therein involved. In particular, once emptied a siphon will never be full again. This explains why a siphon is sometimes also called a “locking set” in the Petri net literature. A precise statement of the foregoing remarks is as follows.

**Proposition 5.5** Let  $Z \subset \mathcal{S}$  be non-empty. Then  $Z$  is a siphon if and only if  $\text{cl}(L_Z)$  is forward invariant for (6).

*Proof. Sufficiency:* Pick  $\xi \in L_Z \neq \emptyset$ . Then forward invariance of  $\text{cl}(L_Z)$  implies that  $\Gamma R(\xi) \in TC_\xi(\text{cl}(L_Z)) = TC_\xi(L_Z)$ , where the last equality holds since  $\xi \in L_Z$ . It follows from Lemma 5.3 that  $Z$  is a siphon.

*Necessity:* Pick  $\xi \in \text{cl}(L_Z)$ . This implies that  $\xi_i = 0$  for all  $i \in Z \cup Z'$ , where  $Z' \subset \mathcal{S}$  could be empty. By the characterization of forward invariance of closed sets in terms of tangent Bouligand cones, it suffices to show that  $[\Gamma R(\xi)]_i = 0$  for all  $i \in Z$ , and that  $[\Gamma R(\xi)]_i \geq 0$  for all  $i \in Z'$  whenever  $Z' \neq \emptyset$ . Now by (6),

$$[\Gamma R(\xi)]_i = \sum_k \beta_{ki} R_k(\xi) - \sum_l \alpha_{li} R_l(\xi) = \sum_k \beta_{ki} R_k(\xi) - 0 \geq 0, \quad (11)$$

which already proves the result for  $i \in Z'$ . Notice that the second sum is zero because if  $\alpha_{li} > 0$ , then species  $i$  is a reactant of reaction  $l$ , which implies that  $R_l(\xi) = 0$  since  $\xi_i = 0$ . So we assume henceforth that  $i \in Z$ . We claim that the sum on the right side of (11) is zero. This is obvious if the sum is void. If it is non-void, then each term which is such that  $\beta_{ki} > 0$  must be zero. Indeed, for each such term we have that  $R_k(\xi) = 0$  because  $Z$  is a siphon. This concludes the proof of Proposition 5.4.  $\blacksquare$

## Proof of Theorem 2

Let  $\xi \in \text{int}(\mathcal{O}_+)$  be arbitrary and let  $\Omega$  denote the corresponding  $\omega$ -limit set  $\Omega = \omega(\xi)$ . We claim that the intersection of  $\Omega$  and the boundary of  $\mathcal{O}_+$  is empty.

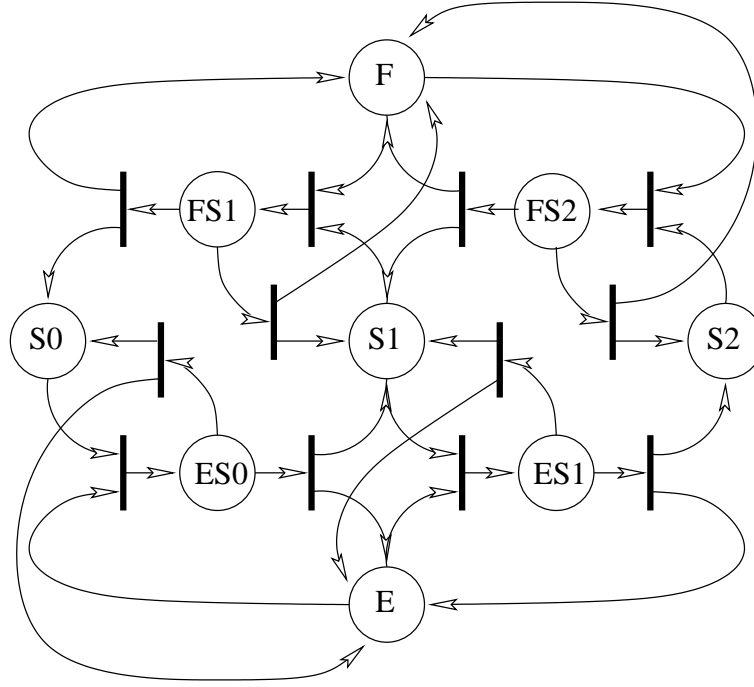


Figure 2: Petri net associated to reactions (2).

Indeed, suppose that the intersection is nonempty. Then,  $\Omega$  would intersect  $L_Z$ , for some  $\emptyset \neq Z \subset \mathcal{S}$ . In particular, by Proposition 5.4,  $Z$  would be a siphon. Then, by our second assumption, there exists a non-negative first integral  $cS$ , whose support is included in  $Z$ , so that necessarily  $cS(t_n, \xi) \rightarrow 0$  at least along a suitable sequence  $t_n \rightarrow +\infty$ . However,  $cS(t, \xi) = c\xi > 0$  for all  $t \geq 0$ , thus giving a contradiction. ■

## 6 Applications

We now apply our results to obtain persistence results for variants of the reaction (b) shown in Figure 1 as well as for cascades of such reactions.

### 6.1 Example 1

We first study reaction (2). Note that reversible reactions were denoted by a “ $\leftrightarrow$ ” in order to avoid having to rewrite them twice. The Petri net associated to (2) is shown in Fig. 2. The network comprises nine distinct species, labeled  $S_0$ ,  $S_1$ ,  $S_2$ ,  $E$ ,  $F$ ,  $ES_0$ ,  $ES_1$ ,  $FS_2$ ,  $FS_1$ . It can be verified that the Petri net in Fig. 2 is indeed consistent (so it satisfies the necessary condition). To see this, order the species and reactions by the obvious order obtained when reading (2) from left to right and from top to bottom (e.g.,  $S_1$  is the fourth species and the reaction  $E + S_1 \rightarrow ES_1$  is the fourth reaction). The construction of the matrix  $\Gamma$  is now clear, and it can be verified that  $\Gamma v = 0$  with

$v = [2\ 1\ 1\ 2\ 1\ 1\ 2\ 1\ 1\ 2\ 1\ 1]'$ . The network itself, however, is not weakly reversible, since neither of the two connected components of (2) is strongly connected. Computations show that there are three minimal siphons:

$$\{E, ES_0, ES_1\},$$

$$\{F, FS_1, FS_2\},$$

and

$$\{S_0, S_1, S_2, ES_0, ES_1, FS_2, FS_1\}.$$

Each one of them contains the support of a P-semiflow; in fact there are three independent conservation laws:

$$E + ES_0 + ES_1 = \text{const}_1,$$

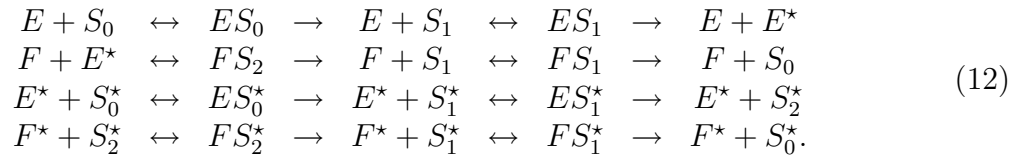
$$F + FS_2 + FS_1 = \text{const}_2, \text{ and}$$

$$S_0 + S_1 + S_2 + ES_0 + ES_1 + FS_2 + FS_1 = \text{const}_3,$$

whose supports coincide with the three mentioned siphons. Since the sum of these three conservation laws is also a conservation law, the network is conservative. Therefore, application of Theorem 2 guarantees that the network is indeed persistent.

## 6.2 Example 2

As remarked earlier, examples as the above one are often parts of cascades in which the product (in MAPK cascades, a doubly-phosphorilated species)  $S_2$  in turn acts as an enzyme for the following stage. One model with two stages is as follows (writing  $S_2$  as  $E^*$  in order to emphasize its role as a kinase for the subsequent stage):



The overall reaction is shown in Fig. 3. Note – using the labeling of species and reaction as in the previous example – that  $\Gamma v = 0$  with  $v = [v_1' \ v_1' \ v_1' \ v_1']'$  and  $v_1 = [2\ 1\ 1\ 2\ 1\ 1]'$ , and hence the network is consistent. There are five minimal siphons for this network, namely:

$$\{E, ES_0, ES_1\},$$

$$\{F, FS_2, FS_1\},$$

$$\{F^*, FS_2^*, FS_1^*\},$$

$$\{S_0^*, S_1^*, S_2^*, ES_0^*, ES_1^*, FS_2^*, FS_1^*\},$$

and

$$\{S_0, S_1, E^*, ES_0, ES_1, FS_2, FS_1, ES_0^*, ES_1^*\}.$$

Each one of them is the support of a P-semiflow, and there are five conservation laws:



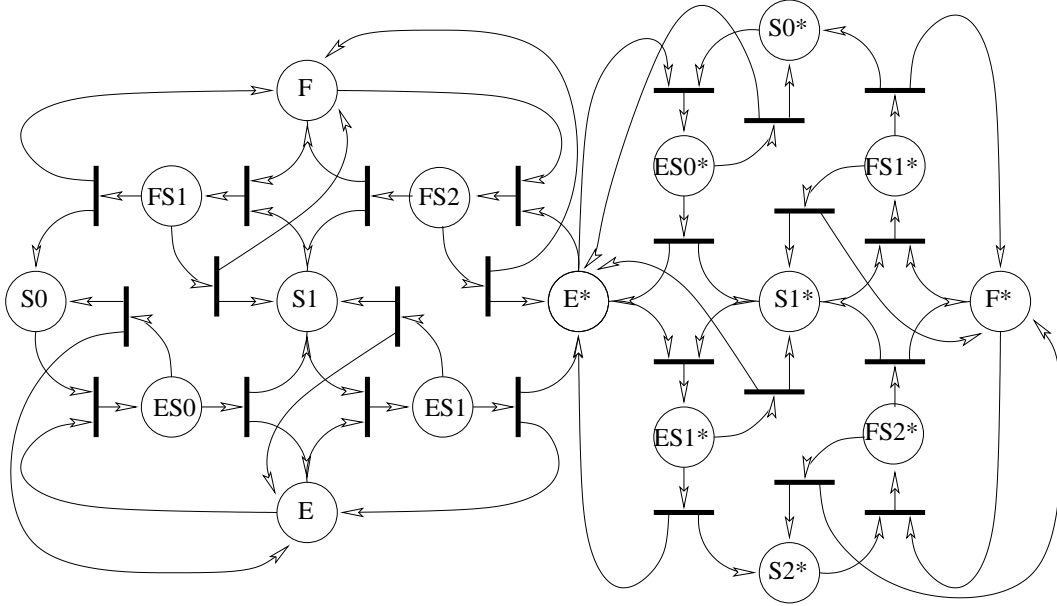


Figure 3: Petri net associated to reactions (12).

$$E + ES_0 + ES_1 = \text{const}_1,$$

$$F + FS_2 + FS_1 = \text{const}_2,$$

$$F^* + FS_2^* + FS_1^* = \text{const}_3,$$

$$S_0^* + S_1^* + S_2^* + ES_0^* + ES_1^* + FS_2^* + FS_1^* = \text{const}_4,$$

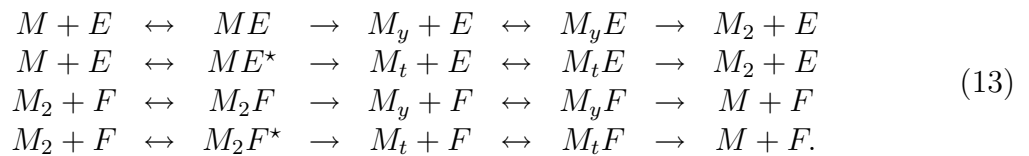
and

$$S_0 + S_1 + E^* + ES_0 + ES_1 + FS_2 + FS_1 + ES_0^* + ES_1^* = \text{const}_5.$$

As in the previous example, the network is conservative since the sum of these conservation laws is also a conservation law. Therefore the overall network is persistent, by virtue of Theorem 2.

### 6.3 Example 3

An alternative mechanism for dual phosphorylation in MAPK cascades, considered in [27], differs from the previous ones in that it becomes relevant in what order the two phosphorylations occur. (These take place at two different sites, a threonine and a tyrosine residue). The corresponding network can be modeled as follows:



See Fig. 4 for the corresponding Petri net. This network is consistent. Indeed,  $\Gamma v = 0$

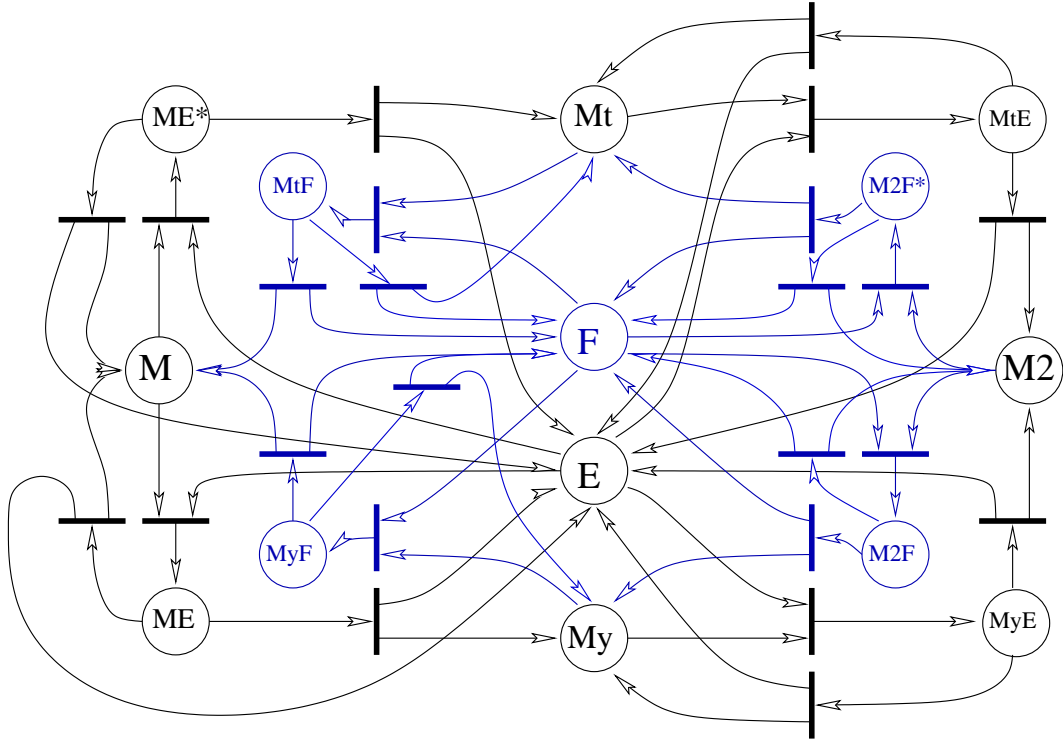


Figure 4: Petri net associated to the network (13).

for the same  $v$  as in the previous example. Moreover it admits three siphons of minimal support:

$$\{E, ME, ME^*, M_yE, M_tE\},$$

$$\{F, M_yF, M_tF, M_2F, M_2F^*\},$$

and

$$\{M, ME, ME^*, M_y, M_t, M_yE, M_tE, M_2, M_2F, M_2F^*, M_tF, M_yF\}.$$

Each of them is also the support of a conservation law, respectively for  $M, E$  and  $F$  molecules. The sum of these conservation laws, is also a conservation law and therefore the network is conservative. Thus the Theorem 2 again applies and the network is persistent.

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